

Original Research Article

<https://doi.org/10.20546/ijcmas.2020.912.083>

Evaluation of Antagonistic Potential of *Bacillus* spp. and *Trichoderma* spp. against *Sclerotium rolfsii* Sacc. causing Collar Rot Disease in *Solanum melongena* L.

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A B S T R A C T

Keywords

Solanum melongena, collar rot, *Sclerotium rolfsii*, *Bacillus* spp., *Trichoderma* spp., Dual culture technique

Article Info

Accepted:
07 November 2020
Available Online:
10 December 2020

Collar rot caused by *Sclerotium rolfsii* Sacc. is one of the catastrophic diseases of brinjal causing in negligible yield loss globally. The present study investigated the effect of *Bacillus* spp. and *Trichoderma* spp. on the growth of collar rot pathogen *S. rolfsii* under *in vitro*. The highest disease incidence was noticed in Vilangudi village which recorded 42.4% followed by Thirumangalam village (39.5%) and least incidence was noticed in Kottampatty village (8.8%) of Madurai district in Tamil Nadu. Results showed that the mycelial growth of the *S. rolfsii* was significantly inhibited by the *Trichoderma* isolate T-AG with 84.44 per cent growth reduction followed by the *Bacillus* isolate B-VI with 55.55 per cent growth reduction.

Introduction

Brinjal (*Solanum melongena* L.) shares many other names such as eggplant/aubergine and belongs to the Solanaceae family. It is originated in the Indian subcontinent and China as reported by (Martin and Rhodes,

1979). It is one of the principal renowned crops acclimatizing tropics and subtropics. It fits into distinct agro climatic zones all-round the year proving its versatility (Singh *et al.*, 2014). India is recorded with the production of 12,779.54 thousand tonnes of Brinjal. Correspondingly, Tamil Nadu occupies

eleventh place with regard to production of brinjal in India (Apeda, 2017-18). Brinjal is cultivated under an area of 728.00 thousand hectares resulting for an yearly yield of 12,660.00 thousand metric tonnes and productivity of 17.7 metric tonnes per hectare (Indiastat, 2018-19). Brinjal extracts have been reported to victoriously quash the growth and development of tumours, lung cancer (Matsubara *et al.*, 2005), inhibit inflammation (Keli *et al.*, 1996) and cardiovascular diseases (Knekt *et al.*, 1996; Knekt *et al.*, 1997). Being a nutrient dense food, it can provide at least 5% of a person's routine requirement of fiber, flavonoids, copper, vitamin B-6 and thiamine. Extracts of the purple skinned brinjal has been shown to exhibit a lofty ability in scavenging superoxide radicals and inhibition of hydroxyl radical production by chelating ferrous iron (Kaneyuki *et al.*, 1999; Noda *et al.*, 2000). All in all, brinjal has received a skyrocketing zest among consumers and researchers throughout and is ranked among top 10 vegetables with regard to antioxidant capacity (Cao *et al.*, 1996). It is decimated by multiple pathogens categorized into fungi, bacteria and viruses. Among fungal diseases, collar rot caused by *Sclerotiumrolfsii*Sacc.is turning out be a profuse menace under nursery and field cultivated brinjal crop. It can infect seeds, seedlings and mature plants in the field, cause diseases to fresh vegetables and rhizomes, while in storage and transit. Collar rot may cause up to 30-50 % loss in fruit yield in eggplant (Siddique *et al.*, 2016). The pathogen invades the collar zone of the host adjacent to the soil level causing death by disrupting translocation of food from top to root zone (Begum *et al.*, 1985). It is a facultative saprophyte and can maintain its generation even under drastic set up by formation of sclerotia. So, it is ineffective and uneconomical to control the pathogen with chemical, which being soil-borne and omnivorous. Hence, biological control which

comprises the employment of various microorganisms to control plant pathogens is seemed to be very beneficial as it may be economically as well as environmentally useful and safer option for modern agriculture practice today. Suryawanshi *et al.*, (2015) reported that *Bacillus megaterium* exhibited the highest mycelial growth inhibition (87.85%) against *S.rolfsii*. Doley and Jite, (2012) reported that the *Trichoderma* isolate inhibited the radial growth of *S. rolfsii* by 75%. Henis *et al.*, (1982) reported mycoparasitism (penetration and infection) of *Trichoderma* spp. against *S.rolfsii*. Jadon and Tiwari, (2011) showed that *T. viridewas* found most effective in inhibiting both mycelial growth (81.2%) and sclerotia production(14.15) of *S. rolfsii*. So keeping this in view, the present study was carried out to study the effect of fungal and bacterial biocontrol agents against the deadly pathogen *S. rolfsii* causing collar rot disease in brinjal.

Materials and Methods

Collection and isolation of *Sclerotiumrolfsii* Sacc.

A survey was conducted in prominent brinjal growing districts of Tamil Nadu. The plants exhibiting collar rot including sclerotial germination which may measure 1-3mm with mustard like appearance upon surfaces of the infected plant parts were collected (Koike, 2004). Then the pathogen was isolated through tissue segment method where the infected tissues along with adjacent small unaffected tissues were cut into small pieces, surface sterilized and plated on potato dextrose agar medium. The infected portion along with healthy portion of plant was cut into small pieces and surface sterilized with 1% Sodium hypochlorite for 1 minute, washed shortly in sterile distilled water and dried on sterile filter paper. The dried pieces were plated onto sterile Petri plate containing

PDA (potato dextrose agar) medium and incubated at $25\pm 1^{\circ}\text{C}$ for seven days. Pure culture of the pathogen was acquired following hyphal tip method and subsequently multiplied on PDA medium in test tubes and Petri dishes and stored at 4°C for further studies (Mian, 1995).

Assessing the virulence of the pathogen

The ten purified isolates of *S.rolfsii* were tested for pathogenicity under *in vivo*. The isolates were mass multiplied in sand maize medium. Sand and ground maize seeds were mixed in the ratio of 19:1, moistened to 50 per cent moisture content and filled in polythene bags. These bags were autoclaved at 1.4 kg/cm^2 pressure for 20 minutes. Then seven days old actively growing mycelial discs (6 mm) of the pathogen isolate was inoculated into each bag under aseptic condition and incubated at room temperature ($28 \pm 2^{\circ}\text{C}$) for 10 days. Then thirty days old brinjal seedlings were transplanted to the pot containing sand maize medium mass multiplied with *S. rolfsii*. Symptoms expression was observed five days after inoculation and percent disease incidence was derived.

$$\text{Percent Disease Incidence} = \left(\frac{\text{Number of plants rotted}}{\text{Total number of plants observed}} \times 100 \right)$$

Isolation of *Bacillus* spp.

The soil samples were collected from rhizosphere region of brinjal crop at various districts of Tamil Nadu. Plant roots adhered with soil was taken for isolation. Before isolation, the roots were gently shaken to remove excess soil and vortexed for 10 min in sterile distilled water (1g per 10 ml). Samples were serially diluted with sterile distilled water from 10^{-1} to 10^{-6} dilutions and one ml of the aliquot from 10^{-5} and 10^{-6} dilutions were plated onto nutrient agar medium (Roy *et al.*,

2007). The isolates were characterised morphologically and biochemically by following Bergey's Manual of Systematic Bacteriology. Then these Isolates were maintained on nutrient agar medium at 4°C for progressive study ahead.

Isolation of *Trichoderma* spp.

The soil samples were collected from the root zones of brinjal crop at different locations in Tamil Nadu. Before isolation, the roots were gently shaken to remove excess soil and vortexed for 10 min in sterile distilled water (1g per 10 ml). Samples were serially diluted with sterile distilled water from 10^{-1} to 10^{-4} dilutions and 1ml of the aliquot from 10^{-3} and 10^{-4} dilutions were plated on *Trichoderma* selective medium. The Petri dishes were rotated clockwise and anticlockwise for uniform distribution and incubated for five days at room temperature ($25 \pm 3^{\circ}\text{C}$). Colonies of *Trichoderma* isolates were identified following a standard key. Then isolates of *Trichoderma* were purified on PDA plates following single hyphal tip technique. After purification, all of the isolates were preserved on PDA slants at 4°C as stock culture for successive use.

In vitro screening of different isolates of *Bacillus* spp. against the mycelial growth of *S.rolfsii*

Eleven isolates of *Bacillus* spp. were screened against the virulent isolate of *S.rolfsii* IS(VIL)-9 under *in vitro* by adapting dual culture technique (Dennis and Webster, 1971). Each bacterial isolate was streaked at one side of the Petri dish containing PDA at one cm away from the periphery of the Petri dish. A nine mm mycelial disc of *S.rolfsii* was placed at a contrary side of the Petri dish at one cm away from the periphery of the Petri dish and perpendicular to the bacterial streak. Control plates were maintained without

bacterial streak. Three replications were maintained at room temperature for four days. After attaining complete growth of the pathogen in the control plate, percent inhibition over control was calculated using the formula proposed by (Pandey and Upadhyay, 2000).

$$PI = \frac{D_c - D_t}{D_c} * 100$$

D_c = average diameter of fungal growth (cm) in control

D_t = average diameter of fungal growth (cm) in treatment

In vitro* screening of different isolates of *Trichoderma* spp. against the mycelial growth of *S.rolfsii

Twelve isolates of *Trichoderma* spp were evaluated in *in vitro* against *S. rolfsii*, applying dual culture technique (Dennis and Webster, 1971). Seven days old cultures of *Trichoderma* spp. and pathogen (*S. rolfsii*) were used for the study. The culture discs (9 mm dia.) of the test pathogen and bioagent were cut out with sterilized cork borer. Then two culture discs, one each of the test pathogen and bioagent were placed aseptically at equidistance and exactly opposite to each other on solidified PDA medium in Petri plates and plates were incubated at $28 \pm 2^\circ\text{C}$. Three replication were maintained. The PDA plate inoculated only with culture disc of test pathogen was maintained as control.

$$PI = \frac{D_c - D_t}{D_c} * 100$$

D_c = average diameter of fungal growth (cm) in control

D_t = average diameter of fungal growth (cm) in treatment

Statistical analysis

Experimental data were statistically analyzed using analysis of variance (ANOVA) and the Statistical Package for the Social Sciences version 16.0. The treatment means were separated at 5% significant level using Duncan's Multiple Range Test (DMRT).

Results and Discussion

Collection and isolation of the pathogen

The data obtained during the survey conducted in major brinjal growing areas of Tamil Nadu was presented in the table 1. Isolates of *S.rolfsii* were isolated from diseased plants showing the typical symptoms of collar rot and presence of sclerotia on the root surface using PDA medium. The fungi were observed under microscope, identified as *S.rolfsii* based on the morphological characters of the fungus and sclerotial structures. The isolated fungus produced dark, white coloured fluffy mycelium and brown sclerotia on the PDA medium. Similarly, Morton, (1969) observed that growth and branching of *S. rolfsii* filamentous fungi occurred at the apex of mycelium and pointed out that growth was regulated by a delicate balance between cell wall synthesis and degradation. Zarani and Christias, (1997); Sarma *et al.*, (2002) reported the production of small, spherical, tan to dark brown and black colored sclerotia in the Petri plates. Thus, the purified fungal culture was sent to the Indian Type Culture Collection, PUSA, IARI, and was confirmed as *Sclerotium rolfsii* with the I.D.No 11,305.20.

Assessing the virulence of the pathogen

The isolated pathogen was proved to be pathogenic on brinjal plants. Inoculated plants produced rotting and disruption in translocating the food from top to root zone

with sclerotia on the root surface. No such symptoms were observed on the uninoculated control plants. Among the different isolates tested, the isolate from Vilangudi was found to be most virulent in inducing the collar rot symptoms. Similarly, Bhuiyan *et al.*, (2012) tested 10 isolates of *S. rolfsii* for their ability

to cause foot and root rot disease of soybean by soil infestation method in pot culture experiment under shade condition. Then the causal agent of pre-emergent seedling mortality was confirmed after re-isolation of the pathogen from un-germinated seeds, infected root and stems.

Table.1 Survey for assessing the collar rot disease incidence in brinjal in different location of Tamil Nadu

S. No	Place of collection	Districts	Isolate code	Geo co ordinates		Percent Disease Incidence(%)
				Latitude	Longitude	
1	Thirumangalam	Madurai	IS (THI)-1	9.8216°N	77.9891°E	39.5
2	Kanakiliyanallur	Trichy	IS (KAN)-2	10.9956°N	78.8800°E	18.8
3	AC & RI	Madurai	IS (AGR)-3	9.9699°N	78.2040°E	10.2
4	Palamedu	Madurai	IS (PAL)-4	10.1043°N	78.1130°E	35.2
5	Vandalaikudalur	Trichy	IS (VAN)-5	10.9701 N	78.8878 E	12.5
6	K.K.Patty	Theni	IS (KKP)-6	9.7386° N	77.3181°E	15.8
7	Pudhupatty	Theni	IS (PUD)-7	9.4356° N	77.9996°E	21.5
8	Ayanpannapatty	Trichy	IS (AYA)-8	9.6313° N	77.7666°E	24.6
9	Vilangudi	Madurai	IS (VIL)-9	9.9498° N	78.0879°E	42.4
10	Kottampatty	Trichy	IS (KOT)-10	10.6228°N	78.4471°E	8.8

Table.2 *Bacillus* spp. isolated from rhizosphere region of brinjal plant in different locations in Tamil Nadu

S. No	Place of collection	Districts	Isolate code	Geo co ordinates		Colony characters
				Latitude	Longitude	
1	Thirumangalam	Madurai	B-TH	9.8216°N	77.9891°E	Dull white colonies
2	Kanakiliyanallur	Trichy	B-KA	10.9956°N	78.8800°E	Regular white colonies
3	AC & RI	Madurai	B-AG	9.9699°N	78.2040°E	Bright white colonies
4	Palamedu	Madurai	B-PA	10.1043°N	78.1130°E	Irregular creamy colonies
5	Vandalaikudalur	Trichy	B-VA	10.9701°N	78.8878 E	Thin white colonies
6	K.K.Patty	Theni	B-KK	9.7386°N	77.3181°E	Regular bright white colonies
7	Pudhupatty	Theni	B-PU	9.4356°N	77.9996°E	Irregular dull white colonies
8	Ayanpannapatty	Trichy	B-AY	9.6313°N	77.7666°E	Light yellow colonies
9	Vilangudi	Madurai	B-VI	9.9498°N	78.0879°E	Flat bright white colonies
10	Kottapatty	Trichy	B-KO	10.6228°N	78.4471°E	Creamy white colonies
11	CR Palayam	Trichy	B-CR	11.2000°N	78.4900°E	Bright white colonies

Table.3 *Trichoderma* spp. isolated from rhizosphere region of brinjal plant indifferent locations of Tamil Nadu

S. No	Place of collection	Districts	Isolate code	Geo co ordinates		Culture characters
				Latitude	Longitude	
1	Thirumangalam	Madurai	T-TH	9.8216°N	77.9891°E	Scattered in dull green
2	Kanakiliyanallur	Trichy	T-KA	10.9956°N	78.8800°E	Dull white to dark green
3	AC & RI	Madurai	T-AG	9.9699°N	78.2040°E	Tufts of white mycelium tuning green
4	Palamedu	Madurai	T-PA	10.1043°N	78.1130°E	Yellowish green tufts
5	Vandalaikudalur	Trichy	T-VA	10.9701 N	78.8878 E	White tufts of mycelium turning into dark green
6	K.K.Patty	Theni	T-KK	9.7386° N	77.3181°E	White tufts of mycelium becoming green from the centre
7	Pudhupatty	Theni	T-PU	9.4356° N	77.9996°E	Dark green in concentric rings
8	Ayanpannapatty	Trichy	T-AY	9.6313° N	77.7666°E	Scattered in minute tufts dark green
9	Vilangudi	Madurai	T-VI	9.9498° N	78.0879°E	Complete dark green
10	Kottapatty	Trichy	T-KO	10.6228°N	78.4471°E	White tufts of mycelium gradually turning into yellowish green
11	CR Palayam	Trichy	T-CR	11.2000°N	78.4900°E	Yellowish green
Ck	TNAU T.V.1 (Check)			11.0152 °N	76.9326°E	White mycelium turning into Yellowish green

Table.4 Efficacy of *Bacillus* spp. against the mycelial growth of *S. rolfsii* IS (VIL)-9 in *in vitro*

S.No.	Treatments	Mycelial growth(cm) [*]	Percent inhibition over control
1	B-TH	6.20	31.11
2	B-KA	6.90	23.33
3	B-AG	6.40	28.88
4	B-PA	5.90	34.44
5	B-VA	5.90	34.44
6	B-KK	6.60	26.66
7	B-PU	6.40	28.88
8	B-AY	5.50	38.88
9	B-VI	4.00	55.55
10	B-KO	4.70	47.77
11	B-CR	5.80	35.55
Control		9.00	
CD(.05)	0.24		

*Mean of three replications

Table.5 Efficacy of *Trichoderma* spp isolates against the mycelial growth of *S.rolfsii* IS(VIL)-9 in *in vitro*

S.No	Treatments	Mycelial growth(cm)*	Percent inhibition over control
1	T-TH	5.00	44.44
2	T-KA	3.70	58.88
3	T-AG	1.40	84.44
4	T-PA	4.40	51.11
5	T-VA	2.60	71.11
6	T-KK	4.10	54.44
7	T-PU	4.20	53.33
8	T-AY	6.10	32.22
9	T-VI	4.20	53.33
10	T-KO	5.10	43.33
11	T-CR	5.20	42.22
Ck	TNAUT.V.1 (Check)	4.10	54.44
Control		9.00	
CD(.05)		0.14	

*Mean of three replications

Smith *et al.*, (1986) proved that *S. rolfsii* isolate from sorghum was found to be pathogenic on greenhouse grown host plants like bean, sugar beet and carrot. Sultana *et al.*, (2012) evaluated *S. rolfsii* for its pathogenicity in a pot culture experiment under the shady atmosphere by soil and seed infestation. Expression of pre-emergence and post-emergence seedling mortality were observed and recorded frequently after sowing. Re-isolation of the pathogen was done to confirm the causal agent of seedling infection as *S. rolfsii*

Isolation and confirmation of biocontrol agents

Eleven isolates of *Bacillus* spp. and twelve isolates of *Trichoderma* spp. were isolated from the rhizosphere region of brinjal plant collected at various places in Tamil Nadu. They were assigned different names as per the biochemical analysis results which confirmed

the fungal isolates as *Trichoderma* spp. and bacterial isolates as *Bacillus* spp. Similarly, *Bacillus* spp. were identified based upon their colony characters, microscopic characteristics and biochemical characters *viz.*, starch hydrolysis (Iverson and Millis, 1974), catalase test (Schaad, 1992) and oxidase test (Gordon and McLeod, 1928). *Trichoderma* spp. were identified based on the morphological features like colony colour, concentric rings with green conidial production, irregular yellow zone without conidia and white pustules on the green mat of conidia depending upon species. *Trichoderma* spp. isolates were identified based on mycological characters.

Effect of antagonistic activity of *Bacillus* spp. against *S. rolfsii* under *in vitro*

Eleven isolates of *Bacillus* spp. were tested against *S. rolfsii* under *in vitro*. Among the isolates, the isolate B-VI draconically inhibited the mycelial growth of *S. rolfsii* (4.0 cm) with

55.55 per cent growth reduction followed by B-KO and B-AY which were succeedingly effective and recorded 4.7 and 5.5cm growth of the pathogen with 47.77 and 38.88per cent growth reduction over control respectively. Similarly, Suryawanshi *et al.*, (2015) reported that *B. Subtilis* and *B. megaterium* were fungistatic against *S. rolfsii*. Suneeta *et al.*, (2017) reported that *B. subtilis* was expressed to be significantly different between antagonistic *Bacillus* strains which proliferated the yield and cut down the collar rot incidence (44.21%) over the control of gerbera collar rot. De Curtis *et al.*, (2010) reported that *B. cepacia* significantly inhibited damping-off caused by *S. rolfsii*, reducing the disease index by 81% compared to the untreated control. Shifa *et al.*, (2015) tested that *B. subtilis* strain G-1 was the most effective in inhibiting the mycelial growth of *S.rolfsii* and recorded an inhibition of 28%. *B. subtilis* secreted antifungal substance which is highly antagonistic against *S. rolfsii* (Nalisha *et al.*, 2006). Gholami *et al.*, (2014) reported that the *Bacillus* sp. and *S. cyaneofuscatus* isolates showed the same capacity for reducing the disease severity of *S. rolfsii* for over 50% (Table 2–4).

Effect of antagonistic activity of *Trichoderma* spp. against *S. rolfsii* under *in vitro*

Twelve isolates of *Trichoderma* spp. and check (TNAU T.v. 1) were tested against *S. rolfsii* under *in vitro* and tabulated in the table 5. Among the isolates, T-AG drastically inhibited the mycelial growth of *S. rolfsii* (1.40 cm) with 84.44 per cent growth reduction followed by T-VA and T-KA which were rigorously effective and recorded 2.60 and 3.70 cm growth of the pathogen with 71.11 and 58.88 per cent growth reduction over control respectively. Similarly, Doley and Jite, (2012) observed that the bioagent *T. Viride* inhibited 75% of *S. rolfsii* growth.

Bhuiyan *et al.*, (2012) showed 83.06% significant reduction of mycelial growth of *S. rolfsii* in presence of *Trichoderma* spp. Madhavi and Bhattiprolu (2011) reported the highest (57.5%) mycelial inhibition of *S. rolfsii* with *T. harzianum* resulting similar to Virupaksha Prabhu *et al.*, (1997) who worked with *T. Harzianum* against collar rot of cotton. Singh and Singh, (1994) proved that *T. harzianum* showed highest antagonistic activity (73%) against *S. rolfsii* of brinjal.

In conclusion there are umpteen results available for the control of *S. rolfsii* under *in vitro* by bioagents like *Bacillus* spp. and *Trichoderma* spp. Present study revealed that the bioagents expressed antifungal activity through competition, antibiosis, mycoparasitism and induced systemic resistance. Moreover, the lofty cost connected with the use of chemical fungicides to control soil borne pathogenic fungi is an extensive limiting factor restraining the profitability of crop production. The use of bioagents in lieu of fungicides or bactericides with high potential is the need of the hour to protect the crop with no harm to environment

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How to cite this article:

Abinaya, A., K. Kalpana, E. G. Ebenezar, S. Thiruvudainambi, M. Theradimani and Arunkumar, R. 2020. Evaluation of Antagonistic Potential of *Bacillus* spp. and *Trichoderma* spp. against *Sclerotium rolfsii* Sacc. causing Collar Rot Disease in *Solanum melongena* L. *Int.J.Curr.Microbiol.App.Sci.* 9(12): 694-703. doi: <https://doi.org/10.20546/ijcmas.2020.912.083>